

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Gen-Ichiro Soma et al.

Application No.: 10/572,853

Confirmation No.: 9265

Filed: February 9, 2007

Art Unit: 1655

For: METHOD FOR FERMENTATION AND
CULTIVATION, FERMENTED PLANT
EXTRACT, FERMENTED PLANT
EXTRACT POWDER, AND COMPOSITION
CONTAINING THE EXTRACT OF
FERMENTED PLANT

Examiner: Q. Mi

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Takashi Nishizawa, hereby declare and state as follows:

1. I am one the inventors of the invention as claimed in the above-identified application, and accordingly, I am familiar with the specification and claims which comprise this application.
2. I am employed at Macrophi Inc. as a chief of the analysis section. I have worked at Macrophi Inc. since July 13, 2006.
3. I received my higher education at Meiji Pharmaceutical University, obtaining a Masters degree in pharmacology, and obtaining a doctorate at Tokyo University.

4. I have also authored several papers, including the following:
- A. Yoshida, A., Inagawa, H., Kohchi, C., Nishizawa, T., Hori, H., and Soma, G-I. *Development of a drug delivery system using a model that mimics chronic infection of Mycobacterium bovis Calmette-Guerin in alveolar macrophages*. Anticancer Res. 27, 3707-11 (2007).
 - B. Inagawa, H., Nishizawa, T., Yoshioka, N., Taniguchi, Y., Kohchi, C., and Soma, G-I. *Preventative and therapeutic potential of lipopolysaccharide derived from edible Gram-negative bacteria to various diseases*. Current Drug Therapy, 3, 26-32 (2008).
 - C. Yoshioka N., Taniguchi Y., Yoshida A., Nakata K., Nishizawa T., Inagawa H., Kohchi C., Soma G-I. *Intracellular localization of CD14 protein in intestinal macrophages*. Anticancer Res. 29, 865-9. (2009)
 - D. Yoshioka N., Taniguchi Y., Yoshida A., Nakata K., Nishizawa T., Inagawa H., Kohchi C., Soma G-I. *Intestinal macrophages involved in the homeostasis of the intestine have the potential for responding to LPS*, 29, 4861-5 (2009).
 - E. In addition, I have authored more than 40 other papers.
5. In particular I performed the bacteria growth observation tests of wheat flour suspension as known by those skilled in the art.
6. I performed the tests and comparative tests in accordance with the methodology set forth in “Nishizawa, T., Inagawa, H., Oshima, H., Okutomi, T., Tsukioka, D., Iguchi, M., Soma, G-I. and Mizuno, D. *Homeostasis as regulated by activated macrophage. I. Lipopolysaccharide (LPS) from wheat flour: isolation, purification and some biological activities*. Chem. Pharm. Bull. 40, 479-483 (1992).”

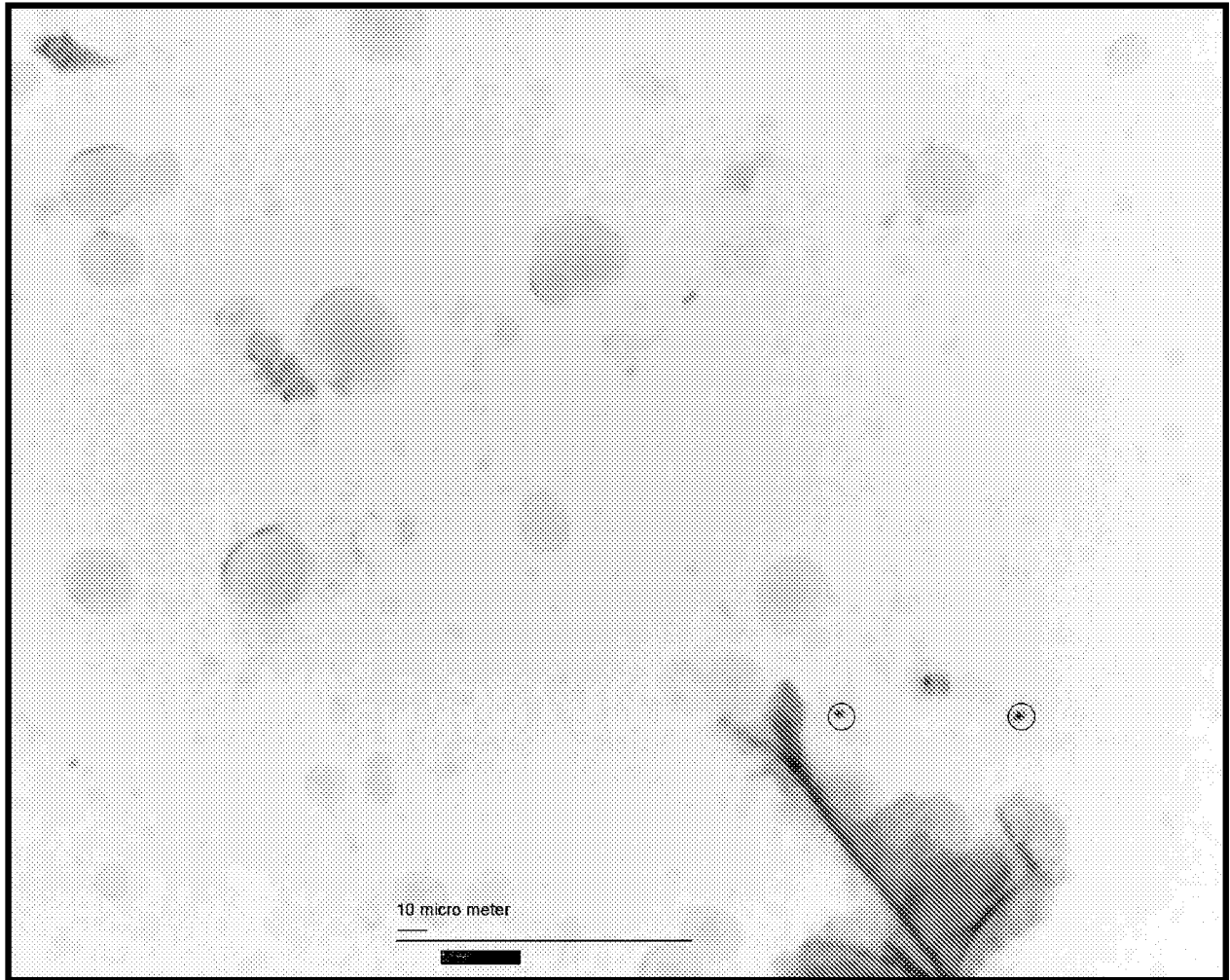
7. The method of the tests and comparative used is as follows:

Number of bacteria in wheat flour incubation* procedure

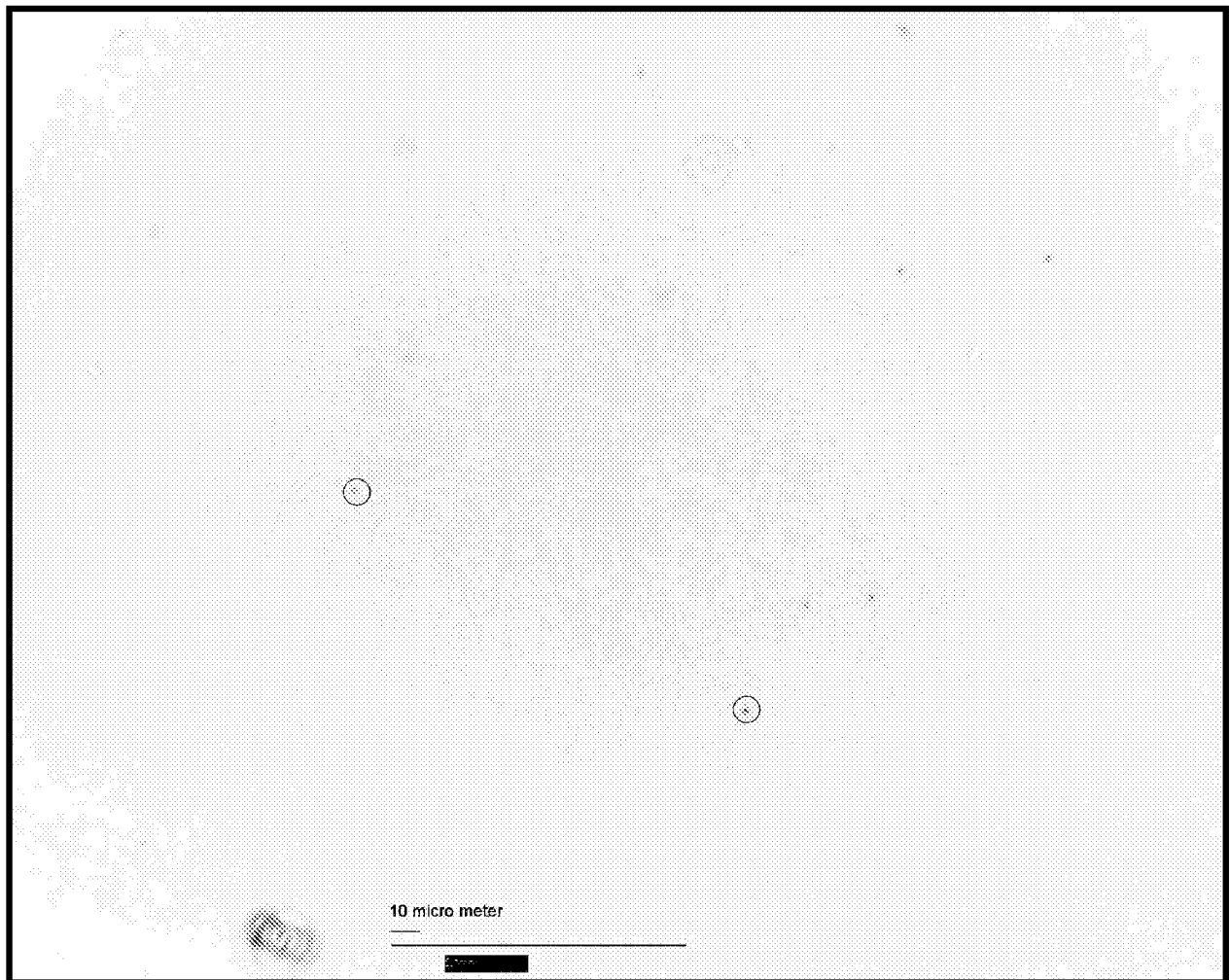
Protocol

- A) In a 50ml corning tube, there was charged 1.04g of hard flour (commercially available wheat flour: Nisshin Co.) followed by addition of 20ml of distilled water thereto to prepare a 50 mg/ml aqueous solution of wheat flour.
- B) The solution was incubated in a water bath at 37°C while shaking, and 0.5 ml portions of the solution were collected at 0, 6, 8 and 45 hours thereafter. For control of the bacteria growth, LB medium** containing *Escherichia coli* (JM109) was cultured in a water bath at 37°C while shaking, and 0.5 ml portions of the solution were collected at 0, 6, 8 hours thereafter.
- C) 2µl portions of the respective solution (sample) was spread over a 10 x 10 mm area on slide glass. The sample was dried by air and fixed by heat, then, stained with DAPI (4',6-diamidino-2-phenylindole) (Invitrogen, SlowFade Gold antifade reagent with DAPI) which binds to DNA. The pictures of the sample were taken by digital camera under fluorescent microscope (Nikon, Eclipse Ti). The number of stained particle (bacterial body) in the sample were counted and calculated as the 1g (wheat flour) or 1ml (LB medium).
- D) The photos below were taken of the Wheat Flour at 6 hours and of Ecoli at 0 and 6 hours. A circle indicates the bacterial body. A bar indicates the 10 micrometer or 0.1mm scale. For counting the number as bacterial body, the particle with fine edge and a length of about 0.5 to 10 micrometer. Counting was done at twelve 0.1×0.1 mm area on the slide.

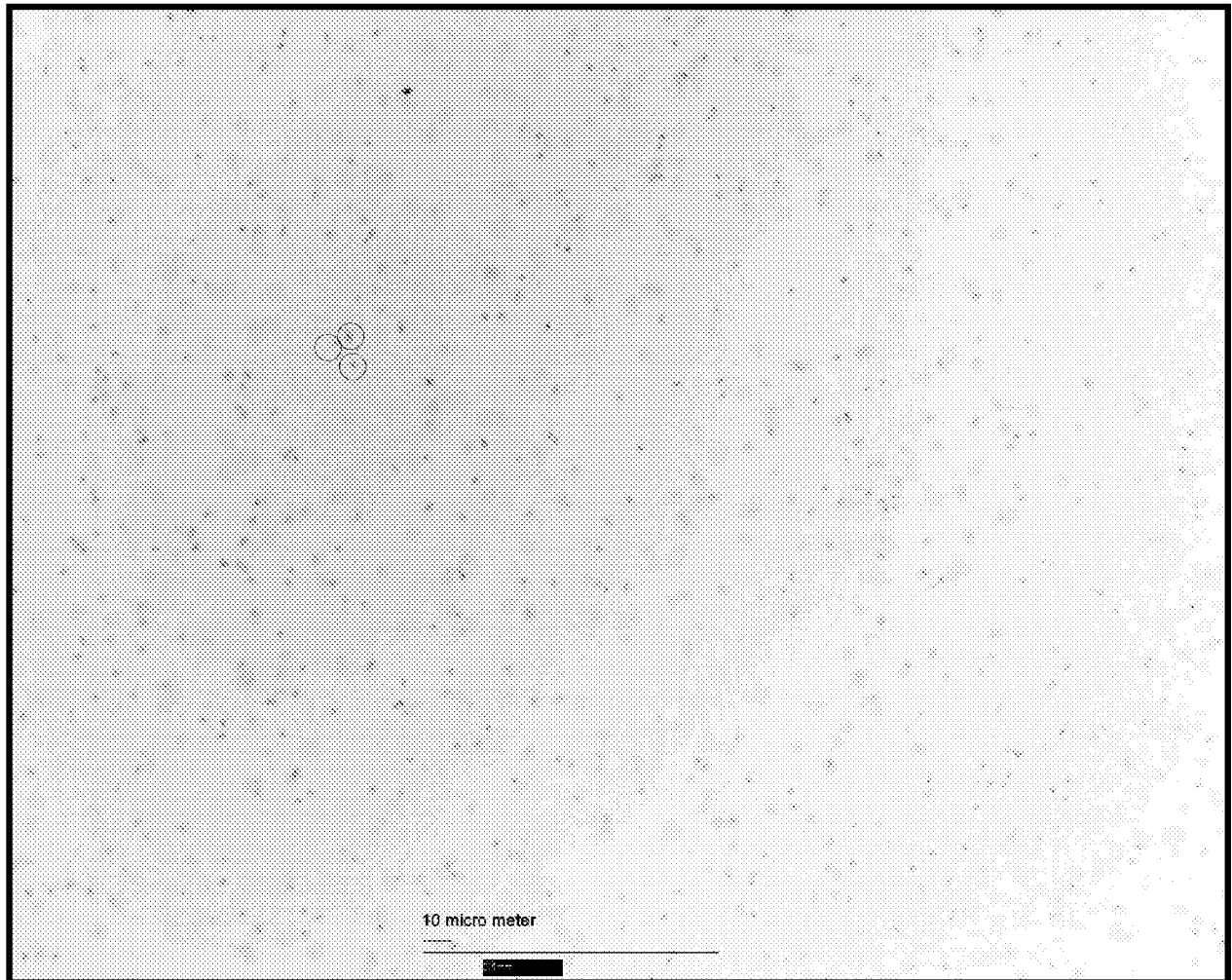
[Wheat flour 6 hours]



[Ecoli 0 hours]



[Ecoli 6 hours]



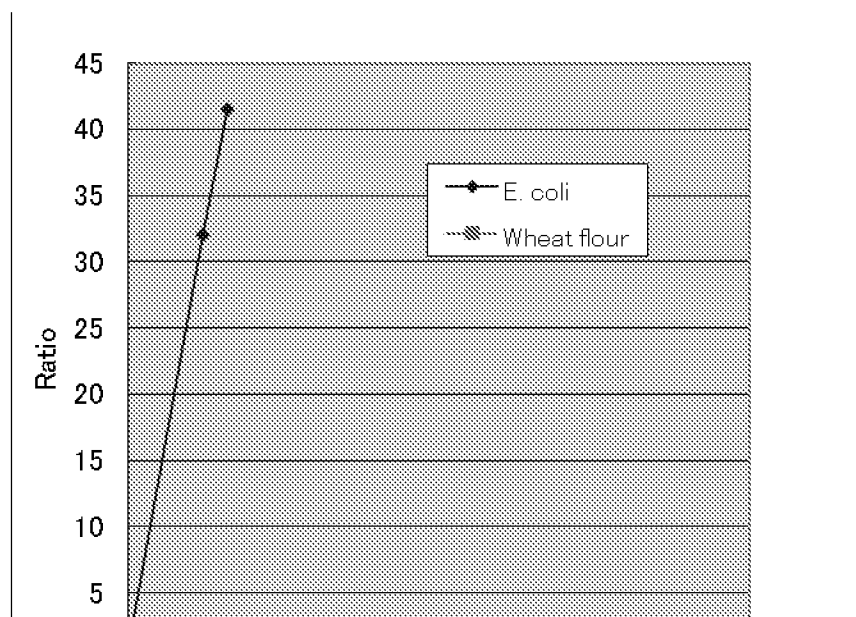
8. The results of the tests are as follows:

The number of bacteria in wheat flour sample and control culture with LB medium were shown in Table 1. The bacterial number in LB medium increased more than 30 times after 6 hours of incubation, but there is no change in the wheat flour sample (Fig. 1).

Table 1. The number of bacteria number in the wheat flour sample

	E. coli in LB medium	Wheat flour
0h	11±6 (million/ml)	18±6 (million/g)
6h	365±20 (million/ml)	8±8 (million/g)
8h	473± 7 (million/ml)	13±5 (million/g)
45h	not tested	18±8 (million/g)

Fig 1. The increasing ratio in wheat flour sample



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